

Transport and Fate of Organophosphate
Insecticides in a Laboratory Model Ecosystem

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Transport and Fate of Organophosphate Insecticides in a Laboratory Model Ecosystem

Jay D. Gile* and James W. Gillett

The disposition of ^{14}C -labeled methylparathion, parathion, and *p*-nitrophenol applied as a foliar spray was examined in the Terrestrial Microcosm Chamber (TMC) and compared to a reference compound, dieldrin. The impact of soil type (synthetic vs. natural) and airflow rates through the chamber on methylparathion disposition were evaluated. The TMC contained either a synthetic soil medium or Willamette sandy loam soil plus agricultural crops, numerous invertebrates, and a gravid gray-tailed vole (*Microtus canicaudus*). Dieldrin was accumulated to a lesser extent than any of the organophosphates in the synthetic soil. Concentrations of methylparathion in the upper layer of Willamette sandy loam soil were consistently lower than those observed with the synthetic medium. Increased airflows altered methylparathion distribution primarily through increased export from the TMC. Although recovery of dieldrin was lower than with equivalent applications of organophosphates, a significantly greater concentration was detected in the vole. Only dieldrin appeared to affect vole survival.

Over the last decade total insecticide production in the United States and domestic consumption increased slowly. However, during that period the market share of organophosphates expanded dramatically because of the increasing restrictions on organochlorine pesticides. Organophosphate insecticides are among the most toxic pesticides in current use (Tucker and Crabtree, 1970; Cholakis et al., 1978). In addition, the term "nonpersistent" as previously applied to organophosphates may no longer be fully appropriate, since a significant proportion of the material applied to a field may become bound in the upper layer of the soil in a form that may retain the active structure (Lichtenstein et al., 1977; Katan and Lichtenstein, 1977). Increased awareness of the potential problems associated with any chemical introduced into the environment either accidentally or purposefully has stimulated new areas of research.

Evaluation of the disposition and effects of pesticides in the face of such complexities has fostered the development of laboratory model ecosystems or microcosms. These simulated ecosystems are gaining increasing acceptance as a safer and more economical approach than field studies for studying the environmental impact of potentially hazardous materials. The simulated ecosystem used in this study was developed at the U.S. EPA's Corvallis Environmental Research Laboratory (Gillett and Gile, 1976).

In experiment I the commonly used organophosphate insecticides methylparathion [*O,O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate], parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate], and a metabolite [*p*-nitrophenol (PNP)] common to both were compared with the organochlorine insecticide dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa-hydro-1,4,5,8-*endo,exo*-dimethanonaphthalene) which serves as a reference compound. In experiment II three treatment levels of methylparathion were studied under environmental conditions different from those of experiment I.

The primary goal of the two experiments discussed in this paper was to evaluate the suitability of the Terrestrial Microcosm Chamber (TMC) for the examination of the

disposition of the organophosphate insecticides within the system and compare this information to the reference compound (dieldrin). Second, the impact of a synthetic soil medium vs. natural topsoil and two different airflow rates on chemical disposition were considered.

MATERIALS AND METHODS

Microcosm. The Terrestrial Microcosm Chamber (TMC; Gillett and Gile, 1976) used in this study consisted of a glass box ($1 \times 0.78 \times 0.61$ m) with a Plexiglas cover. Each chamber received an average of 27 000 lx at the soil surface with a 16-h daily photoperiod. Air temperatures in both experiments ranged from 30 °C during the day to 18 °C at night, except for the dieldrin TMC in experiment I which reached 35 °C during the day. The airflow rate in experiment I was approximately 10 L/min, resulting in two air changes per hour. The synthetic soil medium consisted of 10% (by weight) Jiffy Mix Plus, 45% 20-grit sea sand and 45% illite clay, resulting in an organic matter content of 5%. In experiment II the airflow was increased to 70 L/min (14 air changes per hour) and Willamette sandy loam topsoil (2.12% organic matter, pH 6.4, cation-exchange capacity 19.6) was used. The moisture content in the soil of both experiments was maintained between 15 and 18% water by weight as determined by resistance.

Flora and Fauna. The plants consisted of alfalfa (*Medicago sativa* var. "Thor") and perennial ryegrass (*Lolium perenne*). Bacteriophagic nematodes (*Pristionchus inheriteiri*), earthworms (*Lumbricus* spp.), pill bugs (*Armadillarium* and *Porcellio* spp.), mealworm larvae (*Tenebrio molitor*), gray crickets (*Achetus domesticus*), and garden snails (*Helix aspersa*) represented various invertebrate categories. A gravid gray-tailed vole (*Microtus canicaudus*) was added to represent the top level in the food web.

Radiolabeled Chemicals. The [^{14}C]methylparathion, parathion, and PNP were labeled in positions 2 and 6 of the benzene ring ($\geq 95\%$ purity); [^{14}C]dieldrin was labeled in positions 1, 2, 3, 4, and 10. All chemicals were supplied by Amersham/Searle; gas chromatography was used to verify chemical identity and purity.

Experimental Procedure. The standard experimental procedure in TMC experiments (Gillett and Gile, 1976) was followed in both experiments I and II, except as specifically noted. The soil medium was added to each microcosm in three 6–8-cm layers with each layer com-

* Terrestrial Division, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, Oregon 97330.

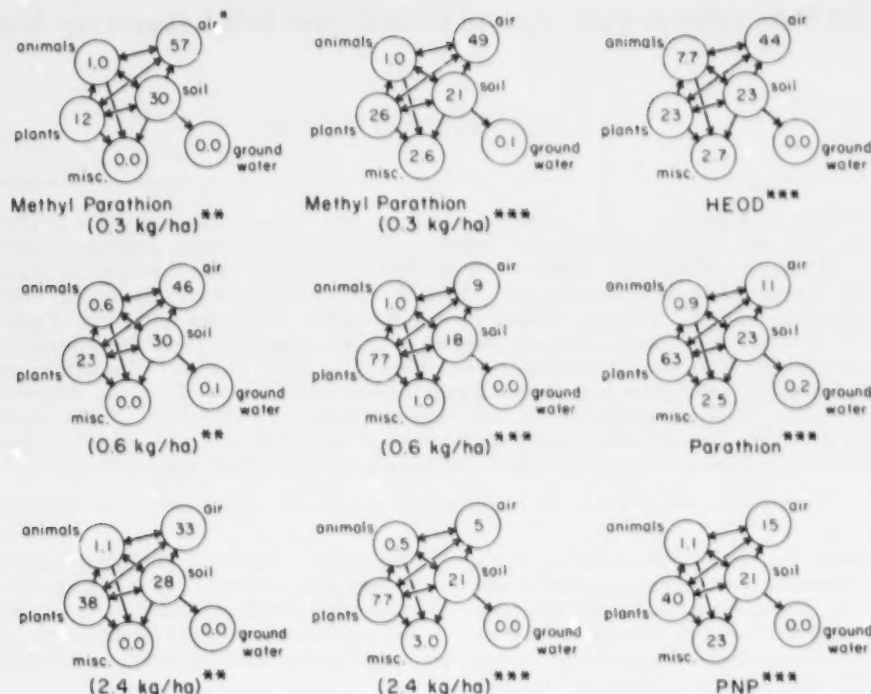


Figure 1. ^{14}C mass balance (percent of applied) in TMC. (*) Amount measured plus estimated loss as $^{14}\text{CO}_2$. (**) Airflow through TMC ~ 70 L/min; Willamette sandy loam topsoil (experiment II). (***) Airflow through TMC ~ 10 L/min; synthetic soil medium (experiment I).

pacted overnight. A total of 60 earthworms and $\sim 20,000$ nematodes were added with the last layer of soil medium. Thirty grams of ryegrass seed and twenty grams of alfalfa seed were planted on opposite longitudinal halves of each microcosm in each experiment. Subsequently, each TMC lid was put in place and the airflow was established at 10 L/min in experiment I and 70 L/min in experiment II; a 16-h daily light cycle was used in both experiments.

Twenty days after planting, crickets, pill bugs, mealworm larvae (100 each), and six snails were added to the microcosms. Ten days later the ^{14}C -labeled chemicals were applied as an aerosol spray. In experiment I dieldrin, parathion, and PNP were applied at the equivalent rate of 0.6 kg/ha (0.5 lb/acre). The methylparathion in both experiments was applied at rates of 0.3 kg/ha (0.25 lb/acre), 0.6 kg/ha (0.5 lb/acre), and 2.4 kg/ha (2.0 lb/acre). Fifty microcuries of ^{14}C -labeled chemical was combined with analytical-grade pesticide to achieve the application rate. A mixture of distilled water, xylene, and emulsifier (Tween 80-Tween 20, 2:1) was used as the carrier for all pesticides. In addition to the treated TMC's, a control (sprayed with adjuvant solution only) was run in concert with each group of TMC's. A gravid vole was added to each TMC 1 week after pesticide application. When a vole died, it was replaced with another gravid female until ~ 20 vole-days had been attained for each TMC.

Visible animals were counted daily. Air samples were taken each day for the first week after pesticide application; thereafter, the air was sampled for ^{14}C 3 times a week. The samples were analyzed for particulates and volatiles trapped on urethane foam but not $^{14}\text{CO}_2$.

At the termination of the TMC, the macrobiota were removed, counted, composited by species, and then frozen until analysis. The soil was removed in four approximately equal layers to determine vertical distribution. After the contents of each TMC were removed, all interior surfaces

were cleaned with acetone and ethanol until free of radioactivity.

Analysis. All analysis for ^{14}C was conducted by the fractionation procedures outlined earlier (Gile et al., 1980), using liquid scintillation spectroscopy. When an organic extract of any soil, plant, or animal tissue contained more than 10^4 dpm of carbon-14, the sample was further analyzed by thin-layer chromatography (TLC) using silica gel G plates (500 μm) with a hexane-ether (1:1) solvent. Materials on the TLC plates were located by radioautography, removed, and analyzed for ^{14}C . Poor separation on the TLC plates prevented a distinction between parent and any metabolites in experiment I. Furthermore, low ^{14}C content did not permit the TLC analysis of many organic samples. The aqueous samples were not analyzed further.

RESULTS

Mass Balance. The ability to recover ^{14}C varied considerably with the chemical under consideration (Table I). Average accountability for methylparathion was 76% ($\pm 25\%$), with values ranging from 51% for the 0.3 kg/ha methylparathion application in experiment II to 100+% for both the 0.6 and 2.4 kg/ha applications of methylparathion in experiment I. Figure 1 illustrates the comparative chemical distribution within each TMC at the termination of the experiment. Of particular interest is the change in ^{14}C materials detected in plant tissue from the two experiments.

Residue Distribution. Soil. Except for the 0.6 kg/ha application in experiment II, most of the ^{14}C -labeled material was found in the upper 5 cm of soil (Figure 2). Concentrations ranged from 0.1 ppm equiv for the 0.3 kg/ha methylparathion treatment in experiment II to 1.33 ppm equiv for the 2.4 kg/ha methylparathion treatment in experiment I. Dieldrin, the only chlorinated hydrocarbon, was present primarily as extractable metabolite (as de-

Table I. ^{14}C (10^6 dpm) Mass Balance

	(a) Synthetic Soil Medium ^b					
				methylparathion		
	dieldrin	PNP	parathion	0.3 kg/ha	0.6 kg/ha	2.0 kg/ha
amount applied	110	116	146	128	117	129
amount recovered	65	103	140	72	124	137
unaccounted dpm ^a	45	13	6	56		
% recovered	59	89	96	56	106	107

	(b) Willamette Topsoil ^c			
	methylparathion			
	0.3 kg/ha	0.6 kg/ha	2.4 kg/ha	
amount applied	138	138	136	
amount recovered	71	99	84	
unaccounted dpm ^a	67	39	52	
% recovered	51	72	62	

^a May reflect conversion of ^{14}C material to $^{14}\text{CO}_2$, which was not detectable by the filtering apparatus, or an extrapolation error as in the case of the 0.6 and 2.4 kg/ha methylparathion treatment in part a. ^b Airflow = 10 L/min. ^c Airflow = 70 L/min.

terminated by TLC), whereas all of the organophosphates were found as bound residues. Concentrations of me-

thylparathion in the upper layer of Willamette top soil were consistently lower than those observed with the synthetic medium.

Plants. Rye samples were not available for analysis in experiment I and only above-ground plant material was analyzed in experiment II. Labeled materials from methylparathion accumulated to a significantly greater degree in experiment I at all treatment levels in comparison to experiment II (Figure 3). Labeled materials from the dieldrin treatment did not accumulate to the same extent as the organophosphates in experiment I. Previous experience with dieldrin (Gile and Gillett, 1979a,b) suggested that it was present primarily as extractable parent; however, separation techniques did not permit that distinction in experiment I. In both experiments parathion and PNP labeled materials appeared as bound residues more frequently than did methylparathion.

Invertebrates. With all the chemicals, juvenile snails accumulated more than adults except for the 0.3 kg/ha methylparathion treatment in experiment I (Table II (a)). At the 2.4 kg/ha treatment in experiment I, residues in crickets were substantially higher than in any other treatment. Pill bugs accumulated dieldrin, PNP, and 2.4 kg/ha methylparathion labeled materials to the similar extent in experiment I. Except for the two highest concentrations of methylparathion in experiment I, *Tenebrio* accumulated little material. The concentrations of methylparathion materials in adult snails followed the same general pattern in both experiments [Table II (b)]. Intermediate cricket values were comparable between the

Table II

(a) Total Pesticide Residues in Macroinvertebrates (Synthetic Soil Medium)							
chemical, ppm							
species	sample day ^b	HEOD	PNP	parathion	methylparathion		
					0.3 kg/ha	0.6 kg/ha	2.4 kg/ha
snail	termination ^c						
adult		10.2	8.2	4.0	2.4	3.3	22.1
juvenile		115.1	70.3	21.7	0.9	96.3	31.3
feces		139.2	7.2	23.1	95.2	10.5	11.3
cricket	1	— ^d	—	—	5.1	4.4	22.5
	2	5.8	12.8	6.4	—	—	—
	6	—	—	—	9.3	—	—
	7	8.4	6.7	3.5	—	—	—
	13	—	—	7.9	—	—	—
	termination	25.1	—	—	0.2	1.3	333
pill bug	termination	30.4	27.5	3.2	2.8	11.1	21
<i>Tenebrio</i>	termination	0.8	5.4	3.3	—	31.8	15.9
worm	termination	—	—	—	1.1	6.5	—

(b) Total ^a Pesticide Residue in Macroinvertebrates (Willamette Topsoil)				
methylparathion, ppm				
species	sample day ^b	0.3 kg/ha	0.6 kg/ha	2.4 kg/ha
snail	7	2.4	2.4	8.5
	15	2.1	1.9	4.8
	21	1.4	2.8	26.2
	termination ^c	0.8	2.1	14.7
juvenile	termination	—	7.0	31.4
cricket	1	1.7	4.5	4.8
	7	0.9	1.4	5.4
	15	0.3	1.2	1.3
	21	—	—	—
	termination	—	0.3	—
pill bug	termination	0.8	2.7	—
<i>Tenebrio</i>	termination	— ^d	0.2	2.2
worm	termination	—	—	26.3

^a Total residue includes extractable parent, metabolites, and bound residue as ^{14}C equivalents of parent (micrograms per gram fresh weight). ^b Number of days after treatment. ^c Termination day for HEOD = 29 days after treatment, PNP = 26 days after treatment, parathion = 22 days after treatment, 0.3 kg/ha MeP = 19 days after treatment, 0.6 kg/ha = 21 days after treatment, and 2.4 MeP = 22 days after treatment. ^d No sample available. ^e Termination day for 0.3 and 0.6 kg/ha = 26 days after treatment and for 2.4 kg/ha = 23 days after treatment.

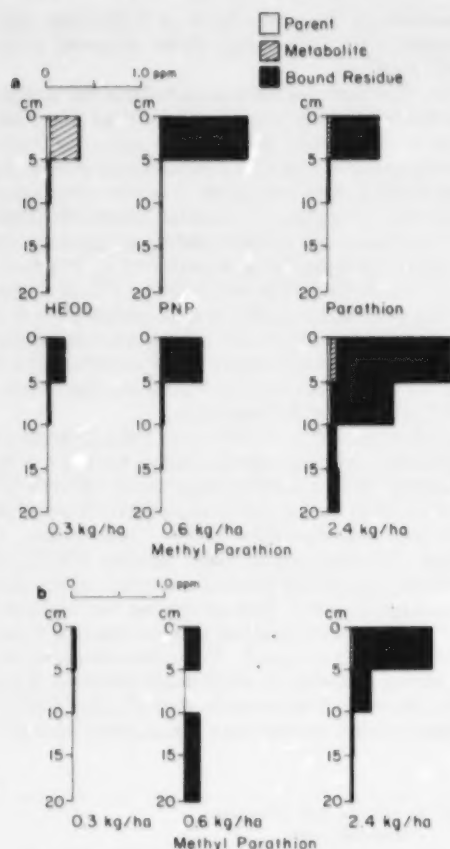


Figure 2. (a) Vertical profile of ^{14}C distribution in synthetic soil medium (experiment I). (b) Vertical profile of ^{14}C distribution in Willamette sandy loam top soil (experiment II).

two experiments; terminal samples were not available for four of the nine treatments. (Note: from previous experience, dead crickets generally disappear quickly from the terraria, leaving only live crickets available for sampling.) Levels in pill bugs and *Tenebrio* were lower in experiment II, while concentrations in earthworms appeared higher. In general, there was an increase in residue levels with increasing methylparathion treatment in both experiments.

Vole. Dieldrin applied at a rate of 0.6 kg/ha resulted in a higher whole body ^{14}C concentration than any of the other treatments except for the 2.4 kg/ha treatment of methylparathion in experiment I (Table III). Of particular importance are the higher residue levels in the brain of the dieldrin voles. PNP material concentrations in experiment I were equivalent to the 0.6 kg/ha methylparathion treatment, while parathion values were substantially lower. Whole body concentrations of methylparathion materials in experiment I were 2–3 times that found in experiment II at all levels.

Air. At the lower airflows in experiment I (10 L/min), ^{14}C methylparathion material losses via the air did not decline with time, although the erratic pattern may have obscured any trend (Figure 4). Dieldrin, PNP, and parathion ^{14}C -labeled materials did exhibit a slight decline with time. With the higher airflow in experiment II (70 L/min), ^{14}C air concentrations at all three treatment levels exhibited a substantial and parallel decline in activity.

DISCUSSION

Mass Balance and Distribution. The recovery of ^{14}C dieldrin materials in experiment I was substantially

Table III. Total ^{14}C Residues^a in Vole

chemical	concn, ppm	
	whole body	brain
Synthetic Soil		
HEOD: ^b vole 1	66.9	3.4
2	104.8	9.9
3	64.0	8.6
PNP	16.4	0.9
parathion	8.5	0.5
methylparathion		
0.3 kg/ha	6.3	0.4
0.6 kg/ha	17.8	0.8
2.4 kg/ha	74.8	5.7
Willamette Topsoil		
methylparathion		
0.3 kg/ha	3.2	0.2
0.6 kg/ha	7.1	0.7
2.4 kg/ha	23.9	2.7

^a Total residue includes extractable parent, metabolites, and bound residue as ^{14}C equivalents of parent (micrograms per gram fresh weight). ^b Three voles serially introduced to the same TMC following the death of the preceding vole; all other TMC's contained only one vole.

Table IV. Vapor Pressures of Test Materials

chemical	vapor pressure, mmHg
dieldrin ^a	7.78×10^{-7} (25 °C)
parathion ^b	0.57×10^{-5} (20 °C)
methylparathion ^c	0.97×10^{-5} (20 °C)

^a Spencer (1973). ^b U.S. Environmental Protection Agency (1975a). ^c U.S. Environmental Protection Agency (1975b).

lower than that of other foliar applications in the TMC (Gile and Gillett, 1979a; Gile et al., 1980). Air temperature in the dieldrin treatment was consistently higher than the other treatments and previous experiments, presumably resulting in an increased vaporization rate and a concomitant increase in air transport out of the TMC. Except for the 0.3 kg/ha treatment, recovery of all organophosphates in experiment I was substantially higher than that found in experiment II. This can be attributed to the difference in airflows between the two tests. The higher airflows in experiment II could increase volatilization from the surface of the plant tissue. Approximately 20% of each the chemicals applied in experiment I was recovered from the soil. From a comparison of the vapor pressures for dieldrin, parathion, and methylparathion (Table IV), one would expect to find a relatively higher concentration of dieldrin remaining in the soil. Even though these values were low, they are still within the range of values encountered in previous TMC experiments using different application methods and rates.

Except for dieldrin, all other chemicals in both studies were present primarily as bound residues in the soil. The authors wish to draw attention to the conspicuous absence of free dieldrin. The R_f for an apparent dieldrin metabolite was 0.8 of the R_f for the dieldrin reference standard and may reflect a chromatographic anomaly. The predominance of the organophosphate bound residues in the soil in these studies is consistent with the findings of Metcalf et al. (1979). However, they detected 20–30% extractable material in their microcosm soil, presumably due to a shorter test period (10 days vs. an average of 24 days). While the average concentration of methylparathion residues in the upper soil layer of experiment I was generally double that observed in experiment II (Figure 2), the relative amount of ^{14}C -labeled materials recovered from

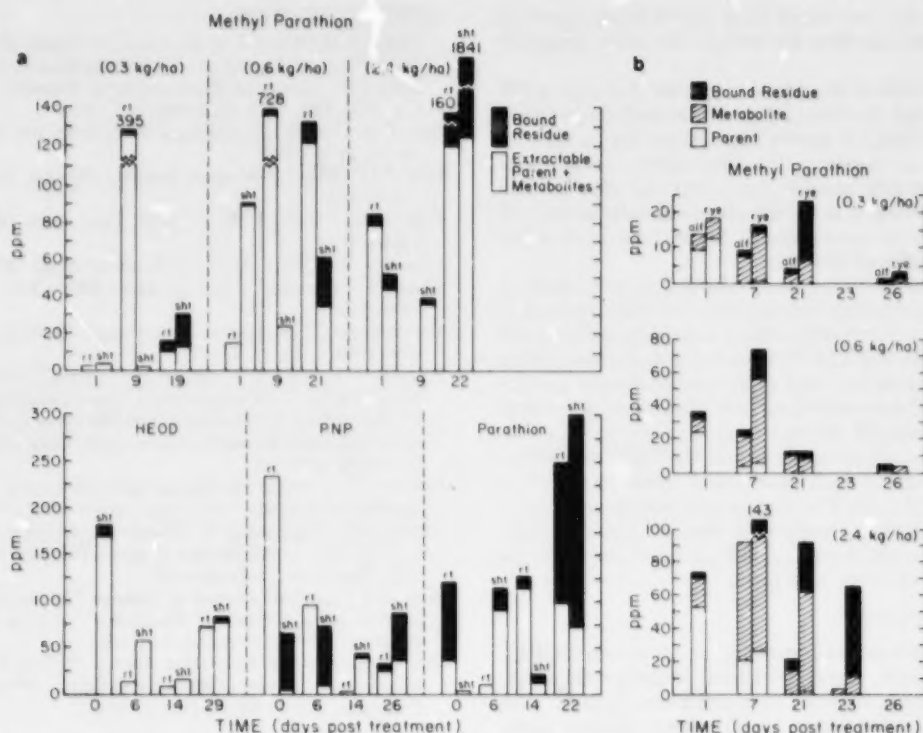


Figure 3. (a) Residue patterns in alfalfa (experiment I). Sh = shoot; rt = root. (b) Residue patterns in rye and alfalfa (experiment II).

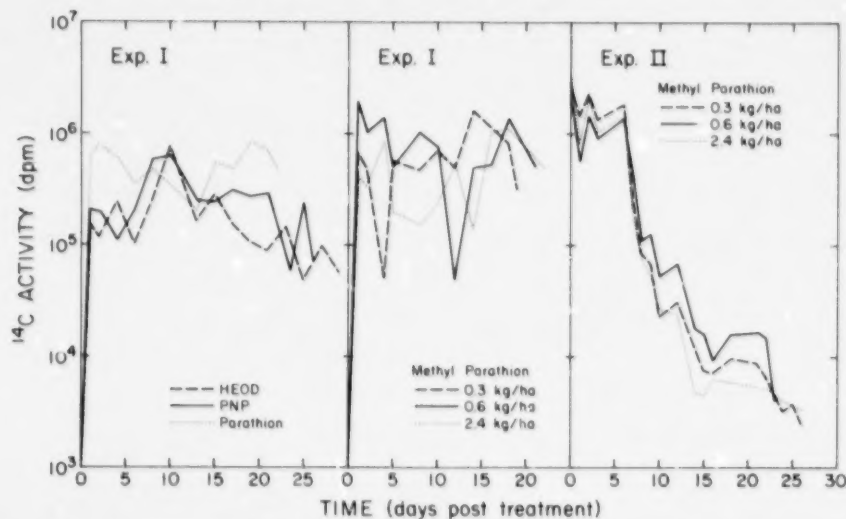


Figure 4. Measured ^{14}C concentrations in air exiting TMC. (Note: initial treatment contained a constant amount of ^{14}C resulting in different specific activities.)

the soil was 10% greater in experiment II in comparison to that of other ecosystem components (Figure 1). The higher organic matter content (5%) of the synthetic soil in experiment I would be expected to bind pesticide residues more effectively than the natural soil (organic content 2%). The qualitative nature of the soil organic carbon in the synthetic soil would be primarily particulate organic carbon (POC) whereas, in addition, in the natural soil one would expect to encounter dissolved organic carbon and polymeric carbonaceous materials. The later two have a more significant role in binding than POC alone, thus accounting for the relatively higher binding in natural soil.

However, the higher relative recovery from the soil in experiment II is not unexpected since more chemical is being volatilized from plant surfaces.

Not only did the plant material in experiment I [and as reported in Metcalf et al. (1979)] contain substantially more [^{14}C]methylparathion materials than plants in experiment II at comparable application rates but also the relative abundance of extractable parent was substantially higher. This can be attributed to the fact that the airflows in experiment I [and Metcalf et al. (1979)] were essentially stagnant in comparison to those in experiment II. As previously indicated, the more rapid airflow in experiment

II was one of the two major factors governing chemical distribution and loss from the system, the other being soil type.

Relatively little of the amount applied for any of the chemicals, except dieldrin (Figure 1), reached the animals. Even though Table II shows high values for certain invertebrates (e.g., crickets), the concentration relative to the application rate remains low. Dieldrin affected vole survival as is evident from the observed mortalities and associated high concentrations of dieldrin materials in the bodies and brains of the voles.

Conclusions. The following conclusions are based on the data from these two experiments. (1) The comparable results for the methylparathion experiments in both studies indicate that the TMC is suitable for examining organophosphates and that reproducible results are obtainable. (2) The increased airflow used in experiment II substantially altered the mass balance and residue distribution of methylparathion within the TMC ecosystem. (3) Soil type affected mass balance and residue distribution within the TMC. (4) The presence of bound residues of the organophosphates suggested that, within the TMC under certain conditions (e.g., low airflow), they may be characterized as fairly persistent.

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